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THE GENETICS OF SEXUAL INCOMPATIBILITY IN THE INDIAN PAINT FUNGUS, *ECHINODONTIUM TINCTORIUM*

A. D. WILSON

Department of Plant Pathology, Washington State University, Pullman, Washington 99164-6430

ABSTRACT

Basidiocarps of *Echinodontium tinctorium* were collected from three widely separated localities in Idaho and Arizona. Freezing basidiospore prints at −20 C for ten weeks stimulated germination. The role of low temperatures in breaking spore dormancy is discussed relative to environmental adaptation. Single-spore isolates of a single basidiocarp from each location were paired separately in all possible combinations. Four mating types were identified from each basidiocarp in Idaho. Four types of macroscopic interactions were observed in pairings between sympatric isolates. Basidiospores and cells of hyphae derived from basidiospores were monokaryotic (uninucleate), lacked clamp connections, and appeared haploid. Heterokaryotic hyphae derived from compatible matings were dikaryotic, exhibited clamp connections, and resembled generative contextual hyphae of basidiocarps. Complete intercompatibility between allopatric isolates demonstrated that the incompatibility factors were multiallelic. The fungus apparently does not fruit in culture, although mating results and cytological data indicate that it is heterothallic with a bifactorial (tetrapolar) mating system in which sexual incompatibility is controlled by multiple alleles at two loci on separate chromosomes.

Key Words: *Echinodontium*, incompatibility, spore germination, wood decay, heartrot, population genetics

Echinodontium tinctorium (Ell. & Ever.) Ell. & Ever., the Indian paint fungus, is considered the most serious cause of heartrot and volume losses in western species of living true firs (*Abies* spp.) and hemlocks (*Tsuga* spp.), particularly in mature and overmature stands (Maloy, **1967**). The fungus is infrequently found on species of *Picea* and *Pseudotsuga*. It occurs sporadically throughout western North America in coniferous forest associations of predominantly cold mountainous areas from Mexico to Alaska. Distribution of *E. tinctorium* does not extend to eastern North America or elsewhere in the world (for taxonomic description, see Gilbertson and Ryvarden, **1986**). Decay develops as a physiological white rot in the heartwood and advances until an extensive decay column predisposes the tree to wind throw or bole break.

Ellis and Everhart (1895) originally described the fungus as *Fomes tinctorius* Ell. & Ever., but after examining more complete specimens, they (Ellis and Everhart, 1900) renamed the fungus *Echinodontium tinctorium*. Several nomenclatural and taxonomic synonyms have been proposed (see Maloy, **1967**), but none were widely accepted. Presently, the species may be monotypic in North America, since *E. ballouii* (Banker) Gross (= *Steccherinum ballouii* Banker), the only other indigenous species recognized, could be extinct (Gilbertson and Ryvarden, **1986**).

Mayers (**1932**) probably described the first attempt to determine the sexuality of *E. tinctorium*. He was able to obtain only a few single-spore isolates because of the low rate of basidiospore germination. He observed that monosporous cultures lacked clamp connections and he paired the isolates in all possible combinations. Only two compatible pairings were observed as indicated by clamp connection formation. Mayers (**1932**) concluded that *E. tinctorium* was a heterothallic species, but he was unable to determine its mating system. Miller (**1962**) also reported an absence of clamp connections on hyphae derived from germinating spores.

Few aspects of the biology, ecology, and life history of *E. tinctorium* are well understood, despite extensive research (Maloy, **1967**). The objectives of this study were to develop a more efficient means of stimulating spore germination, to determine the mating system and nuclear condition during its life cycle, and to investigate intercompatibility between allopatric isolates.

MATERIALS AND METHODS

Fruiting body collections and sporulation.—Single basidiocarps of *E. tinctorium* from three sites were utilized to obtain single-spore isolates for

spore germination and mating studies. The collections were obtained from the following sources and locations: ADW-LP 160 ID on *Abies grandis* (Dougl.) Lindl., near Disalto Creek Trail at the base of Strychnine Ridge and Sandy Mtn., adjacent to Laird Park, St. Joe Nat. Forest, Latah Co., Idaho; ADW-BP 230 ID on *A. grandis,* at Black Pine Cabin Picnic Area near Waha, Craig Mtns., Nez Perce Co., Idaho; and ADW-SH 310 AR on *Abies concolor* (Gord. & Glend.) Lindl., near Summerhaven, Mt. Lemmon, Santa Catalina Mtns., Coronado Nat. Forest, Pima Co., Arizona. The basidiocarps were collected during peak sporulation periods in late September to early November and late March to early May (Maloy, **1961**, **1963**). The three specimens were deposited at the Washington State University Mycological Herbarium (WSP) in Pullman. Accession numbers of the specimens are ADW-LP 160 ID = WSP 69527, ADW-BP 230 ID = 69528, and ADW-SH 310 AR = WSP 69529.

Each basidiocarp was placed on pieces of Saran Wrap stretched over corrugated cardboard frames. Sporulation was induced by placing the basidiocarps under sealed tents of Saran Wrap or plastic bags containing small dishes of distilled water to form high humidity chambers. The chambers were refrigerated at 4 C to minimize contamination of spore prints and prolong sporulation. Sporulation usually commenced within seven days. Spore prints were stored at 4 C under low humidity to minimize respiration of food reserves.

Basidiospore germination induction and homokaryon isolations.—Portions of spore prints taken from basidiocarps collected before freezing temperatures occurred in the field were frozen at either −4 C or at −20 C under low relative humidity for 9–10 weeks. Spore prints which had never been frozen served as controls. Free-casted basidiospores were suspended in sterile distilled H_2O and vigorously vortexed. Spore suspensions were adjusted to known concentrations of 3.5–4.0 × 10^4 spores/ml using a Hausser hemocytometer. This concentration range provided optimum numbers of sufficiently separated spores to allow observations of individual germ tubes. Approximately 2 ml of each spore suspension were plated onto 4.5% Difco malt agar (MA) containing 0.1% streptomycin sulfate in 9.0 cm plastic Petri dishes. The plates were air-dried 1 h, sealed with Parafilm, and incubated at 21 C. Fifteen replicate plates receiving identical spore concentrations and suspension volumes were prepared for each treatment. Fresh spores began germinating within 3–4 days after plating. Germination data were taken microscopically from a 784 mm^2 sample area at 5-day intervals after plating.

Germinating single spores were cut out from the agar surface of spore dilution plates using a fine needle under a 90× stereoscope and transferred to 9.0 cm MA plates. Pure cultures of homokaryotic isolates were stored on MA slants at 4 C and high relative humidity in the dark. Cultures (ATCC 66178–66183) of representative monokaryotic and dikaryotic isolates are available from the American Type Culture Collection.

Mating system, interfertility, and cytological investigations.—Mating studies were conducted by separately pairing homokaryotic isolates of a single basidiocarp from each collection site. Sixty single-spore isolates from ADW-LP 160 ID were divided into six groups of 10 isolates that were each paired in all possible combinations on MA plates and incubated at 22 C under ordinary alternating day and night conditions in the laboratory. Thirty homokaryons from ADW-BP 230 ID and five homokaryons from ADW-SH 310 AR were each paired separately in all possible combinations. Isolates paired against themselves served as controls. All plates were examined macroscopically and microscopically for interactions between opposing colonies 10–12 weeks after pairing. Samples for microscopic study were taken from the contact zones and margins of paired isolates and examined for clamp connections and atypical structures associated with illegitimate or incompatible matings. Dikaryons from compatible pairings were subcultured to determine if the dikaryotic condition was stable. Compatible pairings (+) were recorded only when abundant clamp connections formed in the contact zone. Incompatible pairings (−) were indicated by the presence of few or no clamp connections. Strong aversion zones (barrage reactions) and pseudoclamps were interpreted as interactions between common B alleles (uncommon A) according to convention (Raper, **1966**; Boidin, **1986**). Macroscopic interactions in dimon pairings were recorded for comparison with those observed in pairings of monokaryons.

Tester strains of each mating type (ATCC 66178–66181) from ADW-LP 160 ID were se-

lected and paired in all possible combinations with mating types from each group of ten isolates in the collection to determine the genotypes of all 60 isolates. Similarly, tester strains representing mating type alleles from each collection were paired in all possible combinations to determine the intercompatibility between allopatric isolates, *i.e.,* those originating from different, or divided, geographical localities (Boidin, **1986**).

The nuclear condition of basidiospores and hyphae was investigated using a Giemsa procedure highly modified from Ward and Ciurysek (**1961**). Preparation of slide cultures of homokaryotic and heterokaryotic mycelia followed Riddell (**1950**). When hyphae had grown out sufficiently, they were air-dried for 5–10 min at room temperature. The hyphae were covered with filtered, 0.025% Haupt's adhesive in a 4% formalin solution (Bissing, **1974**) and heated at 40–45 C overnight. The slides were stored indefinitely at this point until processing. Hyphae were hydrated 5–7 min in 60 C distilled H_2O and hydrolyzed 2 min in 5 N HCl at 60 C. The hydration step was omitted for nuclear staining of basidiospores. Basidiospores were hydrolyzed for only 30–60 sec. Slides were subsequently rinsed four times for 1 min intervals each in separate containers of distilled H_2O and in 0.15 M potassium phosphate buffer, pH 6.5 or 7.2. Slides were placed in a 1:25 Giemsa stain-potassium phosphate buffer solution for 30–60 min, rinsed 10 sec in buffer, dipped in 0.01% v/v Tween-20 detergent for 5 sec, and blotted to remove excess moisture around the specimens. Nuclei were differentiated in a 19:1, 14:6, and 6:14 acetone/xylene series for 20–40 sec each followed by three changes of pure xylene. Basidiospores were treated in the acetone/xylene series for only 5 sec at each step to avoid excessive destaining. Permanent slide mounts were prepared with several drops of Coverbond (Harleco) or Permount (Fisher Scientific Co.) resin and heated at 45 C until dry (Wilson, **1988**).

RESULTS

Germination induction.—Germination rates of spores frozen dry at −20 C for 9–10 weeks were significantly greater ($P = 0.05$) than those of spores that had never been frozen. Spores frozen at −20 C germinated at rates that increased from <1.0% after 5 days to >1.5%, 15 days after plating. However, spores frozen at −4 C for the same duration did not show significantly increased germination (0.03%) when compared with the germination rates of spores that were never frozen (<0.01%). More than 90 monosporous cultures were obtained from basidiospores frozen at −20 C.

Mating system and intercompatibility studies.—Pairings between homokaryons of *E. tinctorium* from single-basidiocarp collections at two locations in northern Idaho provided consistent results. All six 10-isolate mating studies of ADW-LP 160 ID showed that each monosporous isolate belonged to one of four mating types and was compatible with only one of the other three mating types. Tester strains, representing the four mating types of ADW-LP 160 ID, were paired with mating types identified from each of the six 10-isolate groups of the same collection. In each case, each isolate was compatible with only one of the four tester strains permitting designations of mating types for all 60 isolates. Results of each 10-isolate study and tester strain crosses of these sympatric isolates were presented previously in more detail (Wilson **1988**). Such mating incompatibility behavior resulted in a typical bifactorial (tetrapolar) pattern. Mating tests with 30 homokaryons from ADW-BP 230 ID resulted in the same pattern with four mating types identified (FIG. 1). However, only five homokaryons representing three mating types were recovered from ADW-SH 310 AR. Nevertheless, retrieval of three mating types among a few spores cast by a single basidiocarp is strong presumptive evidence for a tetrapolar mating system.

Dikaryotic generative hyphae with numerous clamp connections were observed in the contact zone between colonies of compatible mating types 10–12 weeks after plating. Dikaryotic mycelia resulting from compatible matings did not differ macroscopically from the mycelium in colonies of the two mating types from which they were derived. Fruiting bodies were never formed or initiated in the cultures of compatible pairings or from subcultured dikaryotic mycelia derived from compatible matings. Hence, the component mating types could not be recovered from the dikaryons formed in compatible crosses. Heterokaryotic mycelia subcultured from compatible pairings indicated a stable dikaryotic condition. Control pairings (self crosses) of all homokaryotic isolates with themselves were negative for clamp connection formation as were all pairings between isolates of the same mating type.

Four types of macroscopic interactions were

observed between paired colonies of single-spore isolates (FIG. 2A, B). Criteria used to designate specific mating type alleles are summarized in TABLE I. Compatible pairings (A≠B≠) were the only cases where numerous true clamp connections formed in the contact zone and the dikaryotic condition expanded gradually away from the line of contact. Colonies of compatible isolates grew together and freely intermingled with little or no zone of aversion (FIG. 2A). Common A pairings (A=B≠) macroscopically resembled A≠B≠ pairings, although there appeared to be less intermingling of hyphae between the colonies (FIG. 2A). However, clamp connections were not observed in the contact zones of A=B≠ crosses. Common A pairings, in general, did not appear to have any consistent macroscopic interactions, although absence of hyphal massing between paired colonies and reduced aerial hyphae (the "flat" reaction) was occasionally associated with A=B≠ pairings. Likewise, A=B≠ pairings formed simple septa and shared no consistent microscopic characteristics to distinguish them from other interactions. Common B pairings (A≠B=) were usually characterized by a well-developed zone of aversion, commonly referred to as the "barrage reaction" (FIG. 2A), often accompanied by a fine dark reaction line in the aversion zone seen more clearly on the reverse (FIG. 2B). Small numbers of clamp connections and pseudoclamps were found occasionally in the aversion zones of some A≠B= pairings. Pseudoclamps appeared to be hook cells that failed to fuse with the subterminal cell. Two types of macroscopic interactions were associated with A=B= pairings, *i.e.*, between isolates with both A and B alleles in common (FIG. 2A, B): 1) a barrage reaction usually weaker than those observed in A≠B= pairings and lacking a dark reaction line on the reverse (FIG. 2B); and 2) hyphal massing between paired colonies in the contact zone. Hyphal massing is sometimes referred to as the "barrier reaction" in which the massing or hyphae indicates a barrier between interacting colonies that are totally incompatible and of the same mating type. A fine dark reaction line was occasionally observed in association with hyphal massing, but no aversion zone was formed. The dark reaction lines and hyphal massing associated with di-mon pairings were more intense than those observed in A=B= allelic interactions (FIG. 2A, B). Mycelia of single-spore isolates paired against themselves grew together freely without definite signs of interaction.

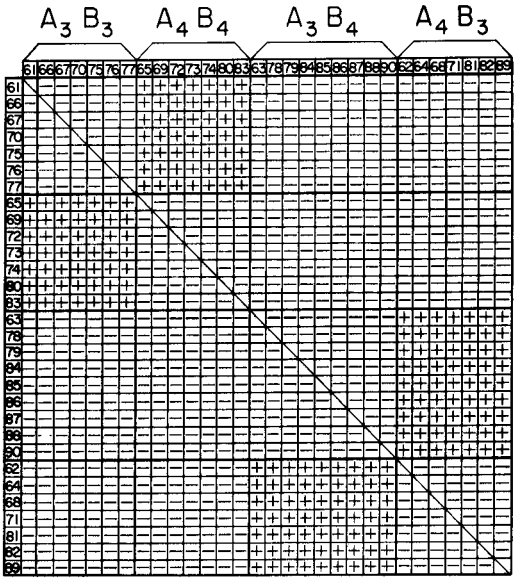

FIG. 1. Mating results for pairings between single-spore (monokaryotic) isolates (61–90) of *E. tinctorium* derived from Idaho collection ADW-BP 230 ID. (+) = compatible reaction; (−) = incompatible reaction.

Tester strains were utilized in intercompatibility crosses between allopatric isolates, representing mating types from the three collections of *E. tinctorium* in Idaho and Arizona, to determine if they were intercompatible and if multiple alleles for incompatibility were present in nature. Although only two mating type alleles were identified at both A and B loci in each collection, all matings between allopatric isolates from the three collections were compatible. Complete intercompatibility demonstrated that the three parental dikaryotic isolates did not share any alleles for incompatibility. Genotypes designated for each isolate from the two Idaho collections are as follows:

ADW-LP 160 ID:
- A_1B_1—1, 2, 6, 10, 14, 19, 21, 22, 24, 31, 33, 36, 43, 45, 49, 58, 60
- A_2B_2—3, 4, 5, 9, 12, 16, 20, 25, 27, 28, 30, 39, 42, 50, 54, 55, 57
- A_1B_2—8, 11, 13, 15, 23, 32, 37, 38, 41, 47, 52, 53
- A_2B_1—7, 17, 18, 26, 29, 34, 35, 40, 44, 46, 48, 51, 56, 59

ADW-BP 230 ID:
- A_3B_3—61, 66, 67, 70, 75, 76, 77
- A_4B_4—65, 69, 72, 73, 74, 80, 83
- A_3B_4—63, 78, 79, 84, 85, 86, 87, 88, 90
- A_4B_3—62, 64, 68, 71, 81, 82, 89

Cytological evidence of nuclear condition.—Most basidiospores of *E. tinctorium* contained a single

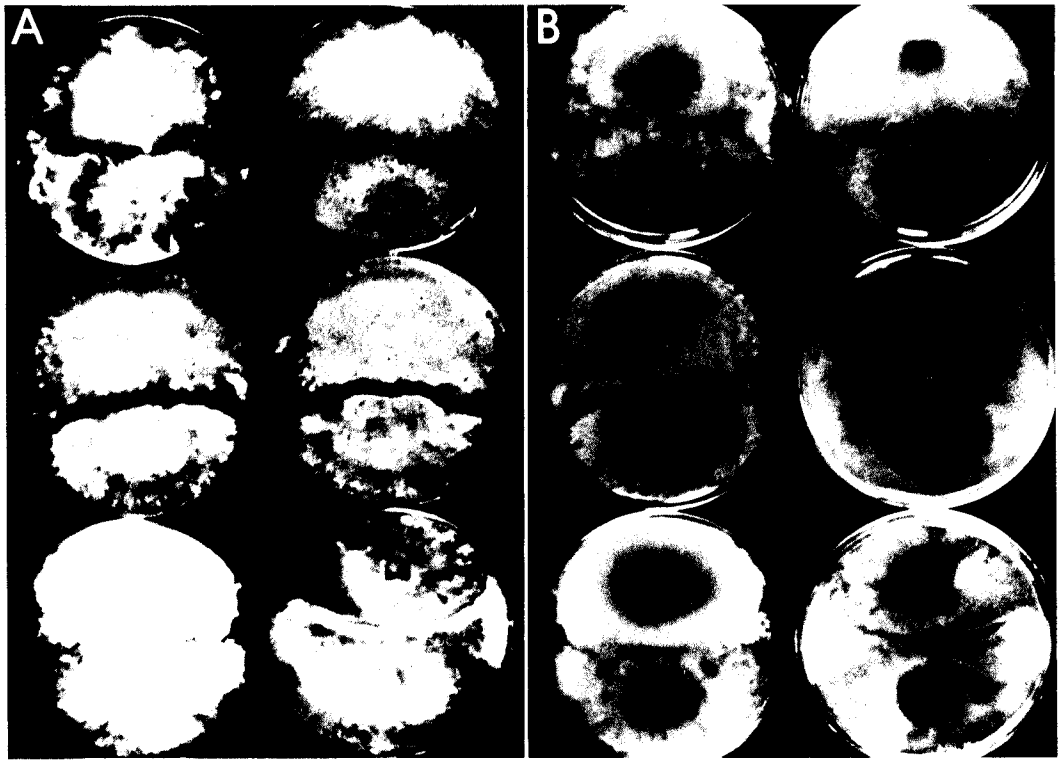

FIG. 2. Macroscopic cultural interactions observed on the front and reverse between paired monokaryons of *E. tinctorium* from ADW-BP 230 ID. A. Front view B. Reverse view. Clockwise from upper left: Compatible reaction (uncommon A&B factors); common A (uncommon B factors) with flat reaction; common B (uncommon A factors) with strong line of aversion (barrage reaction) and fine dark reaction line in contact zone; di-mon interaction with strong hyphal massing and broad darker reaction line in contact zone; common A&B factors (type 2) with mild hyphal massing (barrier reaction) and weak reaction line; common A&B factors (type 1) with weak aversion zone.

nucleus (FIG. 3A). Spores germinated to form a single germ tube through the germ pore at the spore apex (FIG. 3B). The single nucleus within the spore passed through the apical germ pore into the germ tube and initiated mitotic divisions. Resulting hyphae were simple-septate with uninucleate cells (FIG. 3C–F). Occasionally, spores contained two nuclei possibly due to mitosis prior to germination. In such cases, one of the nuclei moved into the germ tube while the other nucleus remained in the spore (FIG. 3E). Nuclear pairs within spores were never observed to initiate conjugate divisions outside the spore. Homokaryotic hyphae from 50 single spores were monokaryotic and lacked clamp connections (FIG. 3F). Young intercalary chlamydospores on homokaryotic hyphae also contained a single nucleus (FIG. 3G). However, nuclei were never seen in the lumina of skeletocystidia and rarely in skeletal hyphae.

Heterokaryotic hyphae from A≠B≠ matings were consistently dikaryotic like generative contextual hyphae of basidiocarps. The dikaryotic condition of hyphae subcultured from 50 A≠B≠ matings for 70 da, was stable. Nuclei appeared filamentous as if migrating or spherical and stationary. As in monokaryotic hyphae, cell length in dikaryotic hyphae was (6–)30–140(–170) μm and nuclei were often widely separated. Clamp connections were lacking at some septa (FIG. 3H), although most septa were clamped (FIG. 3I). Other cells occasionally formed multiple clamps at the septa. Immature intercalary chlamydospores of dikaryotic hyphae were also binucleate (FIG. 3J).

DISCUSSION

Freezing *E. tinctorium* basidiospores as dry spore prints at −20 C for 10 weeks is an effective

TABLE I

CRITERIA USED TO DETERMINE THE MATING TYPE ALLELES OF *ECHINODONTIUM TINCTORIUM*

Interaction	Compatible	Incompatible		
	Uncommon A&B	Common A	Common B	Common A&B
Clamp connections	++[a]	−	(+)	−
Pseudoclamps or degenerated clamps	−	−	(+)	−
Zone of aversion (barrage reaction)	−	−	+ [s]	+ [w]
Reaction line	−	−	+	(+)
Hyphal massing in contact zone (barrier reaction)	−	−	−	+
Flat reaction	−	(+)	−	−

[a] Symbol abbreviations: ++ = consistently present in large numbers; + = very commonly present; (+) = occasionally present; − = absent; [s] = strong reaction; [w] = weak reaction.

means of stimulating spore germination. Large numbers of monokaryotic isolates were obtained efficiently with this procedure despite slow erratic germination at relatively low rates (≤1.8%). Higher rates of germination have been reported to occur on uncolonized hemlock heartwood (Etheridge *et al.*, **1970**) and on agar films, but with spores frozen much longer at −10 C (Etheridge and Craig, **1976**). Exposure to sub-freezing temperatures may play a key role in breaking physiological dormancy. If so, the freezing requirement for spore germination might explain, in part, one means by which *E. tinctorium* has become adapted to cold climate in mountainous areas. The freezing requirement may assure that spores germinate during periods favorable for infection. Thomas (**1958**) showed the incidence of the fungus in British Columbia was highest in high-elevation interior forests with cold winters and lowest in low-elevation coastal forests with mild winters. Very low incidence of the Indian paint fungus west of the Cascade mountain range in the Pacific Northwest is demonstrated by the absence of a report on its occurrence in this region until Foster and Thomas (**1951**) discovered basidiocarps at high elevations on Vancouver Island, but not along the coasts. Sites with mild winter climates might not satisfy the necessary cold requirement to permit adequate levels of germination needed for infections.

Interpretations of allelic interactions in pairing tests of sympatric isolates required both macroscopic and microscopic features. For example, A≠B≠ pairings could only be distinguished from A=B≠ pairings microscopically, *i.e.*, by the presence of true clamps in A≠B≠ pairings. Interactions in A=B≠ crosses lacked consistent macroscopic characteristics, thus A=B≠ crosses were the most difficult to recognize, a feature commonly observed in the bifactorial mating systems of other wood decay fungi. Flat reactions were only occasionally observed in A=B≠ pairings, but they occurred in no other pairing interaction. Common A allelic interactions have long been associated with the flat reaction in *Schizophyllum commune* Fr. (Raper, **1966**). Strong zones of aversion (barrage reactions) conventionally have been interpreted as the result of interactions in A≠B= crosses (Raper, **1966**; Boidin, **1986**). However, the barrage reaction was observed in only 63% of crosses between isolates with B alleles in common. In addition, A=B= pairings frequently formed aversion zones, although milder than in A≠B= pairings, and occasionally formed reaction lines similar to those observed in A≠B= pairings. Interactions in A≠B= pairings were distinguished by the formation of occasional clamp connections and pseudoclamps, while barrier reactions were unique to A=B= pairings. Since A≠B= pairings occasionally produced small numbers of clamp connections, compatible reactions could not be qualitatively based on formation of clamp connections without quantification. A number of mating studies have provided examples where small numbers of clamp connections or pseudoclamps were interpreted as compatible interactions which led to misleading or mixed results (Sass, **1929**, Skolko, **1944**; Whitehouse, **1949**; Esser and Kuenen, **1967**; Setliff, **1970**; Miller, **1971**; Ginns, **1974**). Only observations of large numbers of clamp connections in the contact zones were taken as evidence of a truly compatible mating. Interactions in A=B= pairings were complicated due to formation of two types of reactions, mild barrage reactions and barrier reactions. Common A & B interactions occur between isolates of the same mating type and are most easily identified by

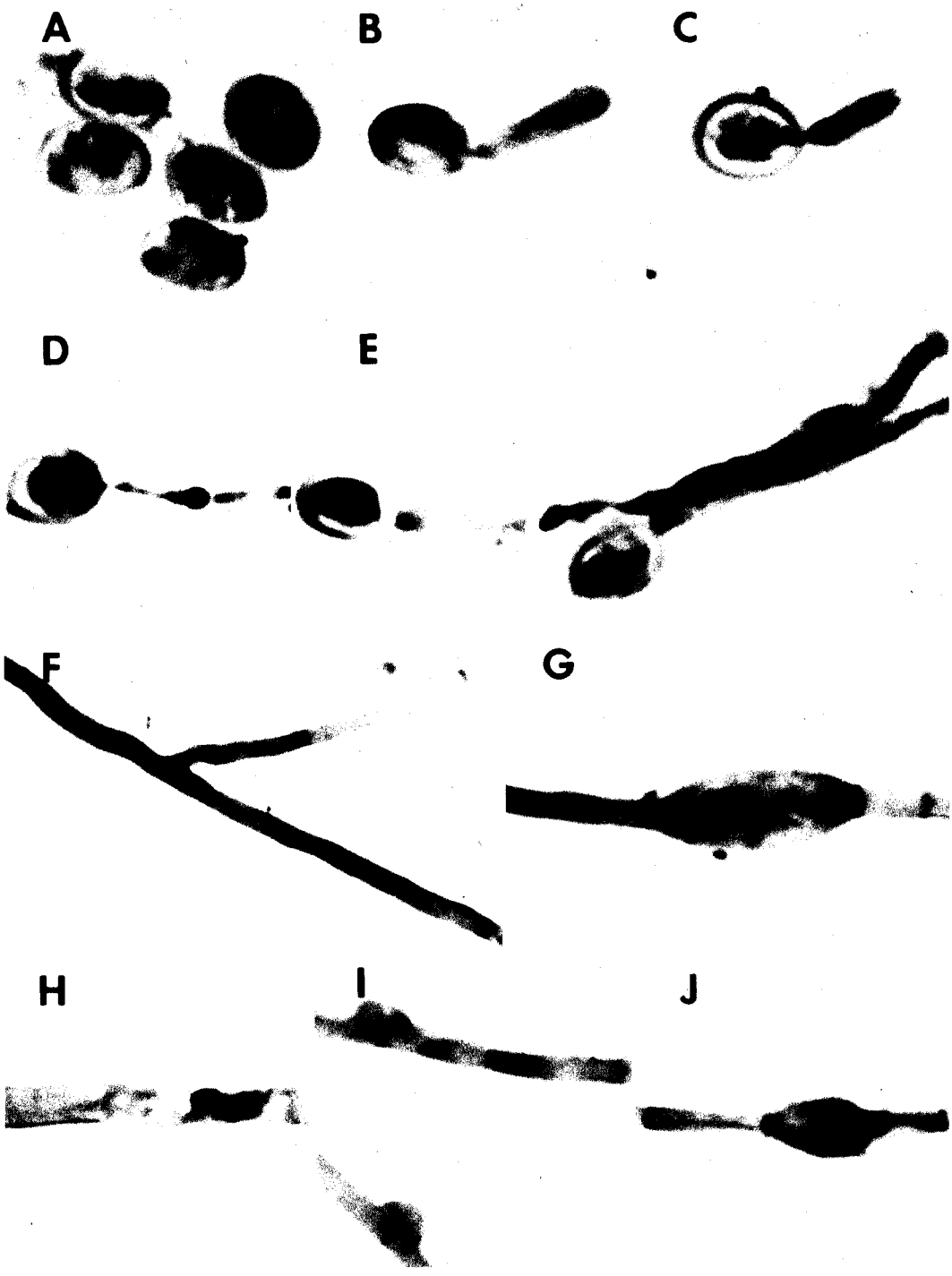

FIG. 3. Nuclear condition of basidiospores, hyphae derived from spores, and hyphae resulting from compatible matings in culture. A–G. Monokaryotic spores and hyphae; H–J. Dikaryotic hyphae and spores. A. Ungerminated basidiospores, ×2353. B. Spore before nuclear migration into germ tube, ×2317. C. Nucleus passing through germ pore into germ tube, ×2370. D. Nucleus migrating through germ tube, ×2141. E. Spores

observing pairings between isolates that have identical mating patterns with other isolates and that are known to be compatible with an identical group of isolates of the opposite mating type.

Formation of abundant clamp connections in A≠B≠ pairings of monokaryons required at least two months to develop after plating. This relatively long time requirement for the mating process is attributed to the slow growth rate of *E. tinctorium* and is longer than the time considered sufficient for the mating process in most other saprobic basidiomycetes (Boidin, **1986**). Occasionally, isolates were obtained which were compatible with two different mating types. These isolates were probably not single-spore isolates, but rather mixtures of two incompatible mating types. Stock cultures of such isolates lacked clamp connections due to incompatibility between the component mating types and hence were not detected until some matings were completed. However, mixed cultures occasionally consisted of mycelia from two compatible mating types which could be detected in stock cultures and control pairings. Mixed cultures were not used in the mating studies, but provided useful checks to ensure that homokaryons were used. Subcultures of dikaryotic hyphae, formed in the contact zones between compatible mating types, confirmed the stability of the dikaryotic condition in A≠B≠ pairings.

Eight distinct mating types were identified among ninety isolates studied from collections at the Laird Park and Waha locations in northern Idaho. The two collection sites were separated geographically by approximately 90 km distance and the Clearwater River Valley. The Arizona collection site was approximately 1600 km from the Idaho sites. Low numbers of monokaryons were obtained from the Arizona site due to insufficient moisture in basidiocarps and contamination of spore prints. As a result, only three of the four mating types at that location were identified. Nevertheless, Papazian test pairings between tester strains (mating types) from each of the three collection sites indicated complete intercompatibility between all allopatric isolates. These results demonstrate that the mating type loci are multiallelic.

Future research is needed to determine the number and geographical distribution of mating alleles. If more extensive collections of *E. tinctorium* basidiocarps were made throughout its wide range and homokaryons from all basidiocarps were paired in all possible combinations, it would be possible to estimate the theoretical number of alleles controlling incompatibility at each locus by extrapolation as shown in the classic paper of Eggertson (**1953**). Determining the number of mating alleles and their distributions may elucidate: 1) the geographical origin of the fungus based on regional incidence of allelic diversity; 2) the number of genetic changes generated by mutational episodes during migration to its present range (and thus an indication of its adaptability); 3) probabilities of mating success and inoculum production expected in each region; and 4) the mechanism(s) of spread within and between forest stands, providing applications in epidemiology. Multiple allelomorphy among allopatric isolates suggests that divergent evolution may have occurred due to geographical isolation. As strains were introduced into new environments, their adaptation to new localized environmental conditions could have included changes in mating incompatibility alleles. Perhaps high mutation rates among incompatibility alleles are stimulated by introductions of strains into drastically different environments miles from the origin of the inoculum. This hypothesis is supported by the wide geographical range of *E. tinctorium* from Mexico to Alaska within which localized populations of the fungus are separated by large distances. Furthermore, the wide range of *E. tinctorium* suggests that the fungus has a means of long-distance dispersal, most likely basidiospores. Based on its wide sporadic distribution, the thick-walled basidiospores of *E. tinctorium* apparently have sufficient longevity and resistance to adverse environmental conditions to serve as long distance dispersal agents of the fungus. Hence, the presence of multiple alleles also implies increased probability for successful outbreeding between allopatric strains which may enhance genetic diversity and adaptability of the species over a wider region.

Echinodontium tinctorium does not fruit

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after mitosis with one nucleus in spore and genetically identical daughter nucleus in germ tube, ×2163. F. Single cell of monokaryotic hypha, ×1160. G. Uninucleate chlamydospore, ×2167. H. Binucleate hypha without clamp connections, ×1677. I. Binucleate hypha with clamp connection, ×1636. J. Binucleate chlamydospore, ×2122.

readily in culture, probably because large decay columns are generally needed to support formation of a basidiocarp. Difficulty of inducing formation of fertile sporophores with viable basidiospores in culture presently precludes establishment of definitive proof of fertility between sympatric isolates and interfertility between allopatric isolates as indicated by Boidin (**1986**). Consequently, the conservative terms "compatibility" and "intercompatibility" are used here instead of "fertility" and "interfertility." Nevertheless, formation of clamp connections and stable dikaryons between paired isolates certainly indicates close intra- and intercompatibility. Although the fungus is not currently known to fruit in culture, all evidence suggests that *E. tinctorium* is heterothallic with a multiallelic, bifactorial (tetrapolar) homogenic mating system in which sexual incompatibility is controlled by multiple alleles at two loci on separate chromosomes. The tetrapolar mating system of this fungus conforms to the pattern of sexuality found in most other white rot fungi (Gilbertson, **1980**). Both monokaryotic and dikaryotic cultures of *E. tinctorium* react positively in phenol-oxidase tests producing a dark brown pigment on the reverse, but they do not grow on gallic or tannic acid media (Davidson *et al.*, **1938**; Nobles, **1948**).

Cytological investigations utilizing Giemsa stain confirmed the nuclear condition of *E. tinctorium* spores and vegetative hyphae at various stages of its life history. Basidiospores were generally uninucleate and appeared to be haploid based on nuclear volume. Some basidiospores contained two nuclei presumed to be genetically identical, postmeiotic products of pregermination mitosis. Such binucleate basidiospores are occasionally produced by a number of wood decay fungi with homogenic incompatibility systems (Ginns, **1974**; Hennon and Hansen, **1987**). Hyphae derived from basidiospores lacked clamp connections and appeared uniformly monokaryotic, while dikaryotic hyphae derived from A≠ B≠ pairings consisted of stable binucleate cells that were perpetuated in subcultures.

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